to evaluate the ability of fusion proteins of the invention to act on hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the $\alpha_5.\beta_1$ and $\alpha_4.\beta_1$ integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and are responsible for stimulating stem cell self-renewal havea not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of 0.2 $\mu g/ cm^2$. Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Albumin fusion proteins of the invention are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where volume of the administed composition containing the albumin fusion protein of the invention represents 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5% CO_2, 7% O_2, and 88% N_2) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

One skilled in the art could easily modify the exemplified studies to test the activity of albumin fusion proteins and polynucleotides of the invention (e.g., gene therapy).

If a particular fusion protein of the present invention is found to be a stimulator of hematopoietic progenitors, the fusion protein and polynucleotides corresponding to the fusion protein may be useful for example, in the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The fusion protein may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the albumin fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention, may also be employed to inhibit the

proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia, since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

Example 39: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation

An albumin fusion protein of the invention is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the fusion protein on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNFa stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100 µl culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5 µg/ml hEGF, 5mg/ml insulin, 1µg/ml hFGF, 50mg/ml gentamycin, 50 µg/ml Amphotericin B, 5%FBS. After incubation at 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50µg/ml Amphotericin B, 0.4% FBS. Incubate at 37 °C until day 2.

On day 2, serial dilutions and templates of an albumin fusion protein of the invention are designed such that they always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml

(AoSMC). Add 1/3 vol media containing controls or an albumin fusion protein of the invention and incubate at 37 degrees C/5% CO_2 until day 5.

Transfer 60μl from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4 degrees C until Day 6 (for IL6 ELISA). To the remaining 100 μl in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10µl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 µl/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 µl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.

Plates are washed with wash buffer and blotted on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100 µl/well. Cover the plate and incubate 1 h at RT. Plates are again washed with wash buffer and blotted on paper towels.

Add 100 μl/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the albumin fusion protein may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of the fusion protein and polynucleotides encoding the albumin fusion protein. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, fusion proteins may be used in wound healing and dermal regeneration, as well as the promotion of vasculogenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, fusion proteins showing antagonistic activity in this assay may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular agent (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for

example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; artheroscleric plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, albumin fusion proteins that act as antagonists in this assay may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

Example 40: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100 μl of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing (containing an albumin fusion protein of the invention) and positive or negative controls are added to the plate in triplicate (in 10 μl volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 μl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10 μl of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10

μg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 μl of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 μl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 μl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 μl of pNNP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 μl of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

Example 41: Alamar Blue Endothelial Cells Proliferation Assay

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37 degreesC overnight. After the overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of an albumin fusion protein of the invention or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is

recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form (i.e., stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity). The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

Example 42: Detection of Inhibition of a Mixed Lymphocyte Reaction

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by fusion proteins of the invention. Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by the albumin fusion proteins that inhibit MLR since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Albumin fusion proteins of the invention found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM®, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to 2 x 10^6 cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to 2 x 10^5 cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of the fusion protein test material (50 µl) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1

μg/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 μg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO_2, and 1 μC of [^{3}H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the fusion protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

Example 43: Assays for Protease Activity

The following assay may be used to assess protease activity of an albumin fusion protein of the invention.

Gelatin and casein zymography are performed essentially as described (Heusen et al., *Anal. Biochem.*, 102:196-202 (1980); Wilson et al., *Journal of Urology*, 149:653-658 (1993)). Samples are run on 10% polyacryamide/0.1% SDS gels containing 1% gelain orcasein, soaked in 2.5% triton at room temperature for 1 hour, and in 0.1M glycine, pH 8.3 at 37°C 5 to 16 hours. After staining in amido black areas of proteolysis apear as clear areas agains the blue-black background. Trypsin (Sigma T8642) is used as a positive control.

Protease activity is also determined by monitoring the cleavage of n-a-benzoyl-L-arginine ethyl ester (BAEE) (Sigma B-4500. Reactions are set up in (25mM$NaPO_4$,1mM EDTA, and 1mM BAEE), pH 7.5. Samples are added and the change in adsorbance at 260nm is monitored on the Beckman DU-6 spectrophotometer in the time-drive mode. Trypsin is used as a positive control.

Additional assays based upon the release of acid-soluble peptides from casein or hemoglobin measured as adsorbance at 280 nm or colorimetrically using the Folin method are performed as described in Bergmeyer, et al., *Methods of Enzymatic Analysis*, 5 (1984). Other assays involve the solubilization of chromogenic substrates (Ward, *Applied Science*, 251-317 (1983)).

Example 44: Identifying Serine Protease Substrate Specificity

Methods known in the art or described herein may be used to determine the substrate specificity of the albumin fusion proteins of the present invention having serine protease activity. A preferred method of determining substrate specificity is by the use of positional scanning synthetic combinatorial libraries as described in GB 2 324 529 (incorporated herein in its entirety).

Example 45: Ligand Binding Assays

The following assay may be used to assess ligand binding activity of an albumin fusion protein of the invention.

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for an albumin fusion protein of the invention is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards the fusion protein. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell polypeptide sources. For these assays, specific polypeptide binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

Example 46: Functional Assay in Xenopus Oocytes

Capped RNA transcripts from linearized plasmid templates encoding an albumin fusion protein of the invention is synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/mi. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocytc) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus oocytes* in response fusion protein and polypeptide agonist exposure. Recordings are made in Ca2+ free Barth's medium at room temperature. The Xenopus system can be used to screen known ligands and tissue/cell extracts for activating ligands.

Example 47: Microphysiometric Assays

Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, Calif.). The CYTOSENSOR is thus capable of detecting the ability of an albumin fusion protein of the invention to activate secondary messengers that are coupled to an energy utilizing intracellular signaling pathway.

Example 48: Extract/Cell Supernatant Screening

A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the albumin fusion proteins of the invention can also be functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts to identify natural ligands for the Therapeutic protein portion and/or albumin protein portion of an albumin fusion protein of the invention. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

Example 49: ATP-binding assay

The following assay may be used to assess ATP-binding activity of fusion proteins of the invention.

ATP-binding activity of an albumin fusion protein of the invention may be detected using the ATP-binding assay described in U.S. Patent 5,858,719, which is herein incorporated by reference in its entirety. Briefly, ATP-binding to an albumin fusion protein of the invention is measured via photoaffinity labeling with 8-azido-ATP in a competition assay. Reaction mixtures containing 1 mg/ml of ABC transport protein are incubated with varying concentrations of ATP, or the non-hydrolyzable ATP analog adenyl-5'-imidodiphosphate for 10 minutes at 4°C. A mixture of 8-azido-ATP (Sigma Chem. Corp., St. Louis, MO.) plus 8-azido-ATP (^{32}P-ATP) (5 mCi/μmol, ICN, Irvine CA.) is added to a final concentration of 100 μM and 0.5 ml aliquots are placed in the wells of a porcelain spot plate on ice. The plate is irradiated using a short wave 254 nm UV lamp at a distance of 2.5 cm from the plate for two one-minute intervals with a one-minute cooling interval in between. The reaction is stopped by addition of dithiothreitol to a final concentration of 2mM. The incubations are subjected to SDS-PAGE electrophoresis, dried, and autoradiographed. Protein bands corresponding to the albumin fusion proteins of the invention are excised, and the radioactivity quantified. A decrease in radioactivity with increasing ATP or adenly-5'-imidodiphosphate provides a measure of ATP affinity to the fusion protein.

Example 50: Phosphorylation Assay

In order to assay for phosphorylation activity of an albumin fusion protein of the invention, a phosphorylation assay as described in U.S. Patent 5,958,405 (which is herein incorporated by reference) is utilized. Briefly, phosphorylation activity may be measured by phosphorylation of a protein substrate using gamma-labeled ^{32}P-ATP and quantitation of the incorporated radioactivity using a gamma radioisotope counter. The fusion portein of the invention is incubated with the protein substrate, ^{32}P-ATP, and a kinase buffer. The ^{32}P

incorporated into the substrate is then separated from free ^{32}P-ATP by electrophoresis, and the incorporated ^{32}P is counted and compared to a negative control. Radioactivity counts above the negative control are indicative of phosphorylation activity of the fusion protein.

Example 51: Detection of Phosphorylation Activity (Activation) of an Albumin Fusion Protein of the Invention in the Presence of Polypeptide Ligands

Methods known in the art or described herein may be used to determine the phosphorylation activity of an albumin fusion protein of the invention. A preferred method of determining phosphorylation activity is by the use of the tyrosine phosphorylation assay as described in US 5,817,471 (incorporated herein by reference).

Example 52: Identification Of Signal Transduction Proteins That Interact With An albumin fusion protein Of The Present Invention

Albumin fusion proteins of the invention may serve as research tools for the identification, characterization and purification of signal transduction pathway proteins or receptor proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as carcinoma tissues, is passed over the column, and molecules with appropriate affinity bind to the albumin fusion protein. The protein complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 53: IL-6 Bioassay

A variety of assays are known in the art for testing the proliferative effects of an albumin fusion protein of the invention. For example, one such asssay is the IL-6 Bioassay as described by Marz *et al.* (*Proc. Natl. Acad. Sci., U.S.A., 95*:3251-56 (1998), which is herein incorporated by reference). After 68 hrs. at 37°C, the number of viable cells is measured by adding the tetrazolium salt thiazolyl blue (MTT) and incubating for a further 4 hrs. at 37°C. B9 cells are lysed by SDS and optical density is measured at 570 nm. Controls containing IL-6 (positive) and no cytokine (negative) are Briefly, IL-6 dependent B9 murine cells are washed three times in IL-6 free medium and plated at a concentration of 5,000 cells per well in 50 μl, and 50 μl of fusion protein of the invention is added. utilized. Enhanced proliferation in the test sample(s) (containing an albumin fusion protein of the invention)

relative to the negative control is indicative of proliferative effects mediated by the fusion protein.

Example 54: Support of Chicken Embryo Neuron Survival

To test whether sympathetic neuronal cell viability is supported by an albumin fusion protein of the invention, the chicken embryo neuronal survival assay of Senaldi *et al* may be utilized (*Proc. Natl. Acad. Sci., U.S.A., 96*:11458-63 (1998), which is herein incorporated by reference). Briefly, motor and sympathetic neurons are isolated from chicken embryos, resuspended in L15 medium (with 10% FCS, glucose, sodium selenite, progesterone, conalbumin, putrescine, and insulin; Life Technologies, Rockville, MD.) and Dulbecco's modified Eagles medium [with 10% FCS, glutamine, penicillin, and 25 mM Hepes buffer (pH 7.2); Life Technologies, Rockville, MD.], respectively, and incubated at 37°C in 5% CO_2 in the presence of different concentrations of the purified fusion protein of the invention, as well as a negative control lacking any cytokine. After 3 days, neuron survival is determined by evaluation of cellular morphology, and through the use of the colorimetric assay of Mosmann (Mosmann, T., *J. Immunol. Methods, 65*:55-63 (1983)). Enhanced neuronal cell viability as compared to the controls lacking cytokine is indicative of the ability of the albumin fusion protein to enhance the survival of neuronal cells.

Example 55: Assay for Phosphatase Activity

The following assay may be used to assess serine/threonine phosphatase (PTPase) activity of an albumin fusion protein of the invention.

In order to assay for serine/threonine phosphatase (PTPase) activity, assays can be utilized which are widely known to those skilled in the art. For example, the serine/threonine phosphatase (PSPase) activity of an albumin fusion protein of the invention may be measured using a PSPase assay kit from New England Biolabs, Inc. Myelin basic protein (MyBP), a substrate for PSPase, is phosphorylated on serine and threonine residues with cAMP-dependent Protein Kinase in the presence of [^{32}P]ATP. Protein serine/threonine phosphatase activity is then determined by measuring the release of inorganic phosphate from 32P-labeled MyBP.

Example 56: Interaction of Serine/Threonine Phosphatases with other Proteins

Fusion protein of the invention having serine/threonine phosphatase activity (e.g., as determined in Example 55) are useful, for example, as research tools for the identification, characterization and purification of additional interacting proteins or receptor proteins, or other signal transduction pathway proteins. Briefly, a labeled fusion protein of the invention is

useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as neural or liver cells, is passed over the column, and molecules with appropriate affinity bind to the fusion protein. The fusion protein -complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 57: Assaying for Heparanase Activity

There a numerous assays known in the art that may be employed to assay for heparanase activity of an albumin fusion protein of the invention. In one example, heparanase activity of an albumin fusion protein of the invention, is assayed as described by Vlodavsky et al., (Vlodavsky et al., Nat. Med., 5:793-802 (1999)). Briefly, cell lysates, conditioned media, intact cells (1 x 10^6 cells per 35-mm dish), cell culture supernatant, or purified fusion protein are incubated for 18 hrs at 37°C, pH 6.2-6.6, with ^{35}S-labeled ECM or soluble ECM derived peak I proteoglycans. The incubation medium is centrifuged and the supernatant is analyzed by gel filtration on a Sepharose CL-6B column (0.9 x 30 cm). Fractions are eluted with PBS and their radioactivity is measured. Degradation fragments of heparan sulfate side chains are eluted from Sepharose 6B at $0.5 < K_{av} < 0.8$ (peak II). Each experiment is done at least three times. Degradation fragments corresponding to "peak II," as described by Vlodavsky et al., is indicative of the activity of an albumin fusion protein of the invention in cleaving heparan sulfate.

Example 58: Immobilization of biomolecules

This example provides a method for the stabilization of an albumin fusion protein of the invention in non-host cell lipid bilayer constucts (see, e.g., Bieri et al., Nature Biotech 17:1105-1108 (1999), hereby incorporated by reference in its entirety herein) which can be adapted for the study of fusion proteins of the invention in the various functional assays described above. Briefly, carbohydrate-specific chemistry for biotinylation is used to confine a biotin tag to an albumin fusion protein of the invention, thus allowing uniform orientation upon immobilization. A 50uM solution of an albumin fusion protein of the invention in washed membranes is incubated with 20 mM NaIO4 and 1.5 mg/ml (4mM) BACH or 2 mg/ml (7.5mM) biotin-hydrazide for 1 hr at room temperature (reaction volume, 150ul). Then the sample is dialyzed (Pierce Slidealizer Cassett, 10 kDa cutoff; Pierce Chemical Co., Rockford IL) at 4C first for 5 h, exchanging the buffer after each hour, and finally for 12 h against 500 ml buffer R (0.15 M NaCl, 1 mM MgCl2, 10 mM sodium phosphate, pH7). Just

before addition into a cuvette, the sample is diluted 1:5 in buffer ROG50 (Buffer R supplemented with 50 mM octylglucoside).

Example 59: Assays for Metalloproteinase Activity

Metalloproteinases are peptide hydrolases which use metal ions, such as Zn^{2+}, as the catalytic mechanism. Metalloproteinase activity of an albumin fusion protein of the present invention can be assayed according to methods known in the art. The following exemplary methods are provided:

Proteolysis of alpha-2-macroglobulin

To confirm protease activity, a purified fusion protein of the invention is mixed with the substrate alpha-2-macroglobulin (0.2 unit/ml; Boehringer Mannheim, Germany) in 1x assay buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM $CaCl_2$, 25 μM $ZnCl_2$ and 0.05% Brij-35) and incubated at 37°C for 1-5 days. Trypsin is used as positive control. Negative controls contain only alpha-2-macroglobulin in assay buffer. The samples are collected and boiled in SDS-PAGE sample buffer containing 5% 2-mercaptoethanol for 5-min, then loaded onto 8% SDS-polyacrylamide gel. After electrophoresis the proteins are visualized by silver staining. Proteolysis is evident by the appearance of lower molecular weight bands as compared to the negative control.

Inhibition of alpha-2-macroglobulin proteolysis by inhibitors of metalloproteinases

Known metalloproteinase inhibitors (metal chelators (EDTA, EGTA, AND $HgCl_2$), peptide metalloproteinase inhibitors (TIMP-1 and TIMP–2), and commercial small molecule MMP inhibitors) may also be used to characterize the proteolytic activity of an albumin fusion protein of the invention. Three synthetic MMP inhibitors that may be used are: MMP inhibitor I, [IC_{50} = 1.0 μM against MMP-1 and MMP–8; IC_{50} = 30 μM against MMP-9; IC_{50} = 150 μM against MMP-3]; MMP-3 (stromelysin-1) inhibitor I [IC_{50} = 5 μM against MMP-3], and MMP-3 inhibitor II [K_i = 130 nM against MMP-3]; inhibitors available through Calbiochem, catalog # 444250, 444218, and 444225, respectively). Briefly, different concentrations of the small molecule MMP inhibitors are mixed with a purified fusion protein of the invention (50μg/ml) in 22.9 μl of 1x HEPES buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM $CaCl_2$, 25 μM $ZnCl_2$ and 0.05%Brij-35) and incubated at room temperature (24 °C) for 2-hr, then 7.1 μl of substrate alpha-2-macroglobulin (0.2 unit/ml) is added and incubated at 37°C for 20-hr. The reactions are stopped by adding 4x sample buffer and boiled immediately for 5 minutes. After SDS-PAGE, the protein bands are visualized by silver stain.

Synthetic Fluorogenic Peptide Substrates Cleavage Assay

The substrate specificity for fusion proteins of the invention with demonstrated metalloproteinase activity may be determined using techniques knonw in the art, such as using synthetic fluorogenic peptide substrates (purchased from BACHEM Bioscience Inc). Test substrates include, M-1985, M-2225, M-2105, M-2110, and M-2255. The first four are MMP substrates and the last one is a substrate of tumor necrosis factor-α (TNF-α) converting enzyme (TACE). These substrastes are preferably prepared in 1:1 dimethyl sulfoxide (DMSO) and water. The stock solutions are 50-500 μM. Fluorescent assays are performed by using a Perkin Elmer LS 50B luminescence spectrometer equipped with a constant temperature water bath. The excitation λ is 328 nm and the emission λ is 393 nm. Briefly, the assay is carried out by incubating 176 μl 1x HEPES buffer (0.2 M NaCl, 10 mM $CaCl_2$, 0.05% Brij-35 and 50 mM HEPES, pH 7.5) with 4 μl of substrate solution (50 μM) at 25 °C for 15 minutes, and then adding 20 μl of a purified fusion protein of the invention into the assay cuvett. The final concentration of substrate is 1 μM. Initial hydrolysis rates are monitored for 30-min.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, patent publications, journal articles, abstracts, laboratory manuals, books, or other disclosures) as well as information available through Identifiers specific to databases such as GenBank, GeneSeq, or the CAS Registry, referred to in this application are herein incorporated by reference in their entirety. The specification and sequence listing of each of the following U.S. applications are herein incorporated by reference in their entirety: Application Nos. 60/229,358 filed on April 12, 2000; 60/199,384 filed on April 25,2000 and 60/256,931 filed on December 21, 2000.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

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Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
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C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
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CANADA

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NORWAY

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AUSTRALIA

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FINLAND

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UNITED KINGDOM

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ATCC Deposit No.: Unassigned

DENMARK

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SWEDEN

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NETHERLANDS

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 58, line 12.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
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C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
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CANADA

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NORWAY

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AUSTRALIA

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FINLAND

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UNITED KINGDOM

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ATCC Deposit No.: Unassigned

DENMARK

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SWEDEN

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NETHERLANDS

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Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
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C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
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CANADA

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FINLAND

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NETHERLANDS

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10801 University Boulevard
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United States of America

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D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
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CANADA

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FINLAND

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UNITED KINGDOM

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NETHERLANDS

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What is claimed:

1. An albumin fusion protein comprising a Therapeutic protein:X and albumin comprising the amino acid sequence of SEQ ID NO:18.

2. An albumin fusion protein comprising a Therapeutic protein:X and a fragment or a variant of the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has albumin activity.

3. The albumin fusion protein of claim 2, wherein said albumin activity is the ability to prolong the shelf life of the Therapeutic protein:X compared to the shelf-life of the Therapeutic protein:X in an unfused state.

4. The albumin fusion protein of claim 2, wherein the fragment or variant comprises the amino acid sequence of amino acids 1-387 of SEQ ID NO:18.

5. The albumin fusion protein of any one of claims 1-4, wherein said Therapeutic protein:X comprises interferon-alpha.

6. An albumin fusion protein comprising a fragment or variant of a Therapeutic protein:X, and albumin comprising the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has a biological activity of the Therapeutic protein:X.

7. The albumin fusion protein of claim 6, wherein said Therapeutic protein:X comprises interferon-alpha, and wherein said fragment or variant has antiviral activity or inhibits cell proliferation.

8. The albumin fusion protein of any one of claims 1-4 or 6, wherein said Therapeutic protein:X, or fragment or variant thereof, comprises a protein selected from the

group consisting of:

(a) serum cholinesterase;

(b) alpha-1 antitrypsin;

(c) aprotinin;

(d) coagulation complex;

(e) von Willebrand factor;

(f) fibrinogen;

(g) factor VII;

(h) factor VIIA activated factor;

(i) factor VIII;

(j) factor IX;

(k) factor X;

(l) factor XIII;

(m) c1 inactivator;

(n) antithrombin III;

(o) thrombin;

(p) prothrombin;

(q) apo-lipoprotein;

(r) c-reactive protein;

(s) protein C; and

(t) immunoglobulin.

9. The albumin fusion protein of any one of claims 1-8, wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the N-terminus of albumin, or the N-terminus of the fragment or variant of albumin.

10. The albumin fusion protein of any one of claims 1-8, wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the C-terminus of albumin, or the C-terminus of the fragment or variant of albumin.

11. The albumin fusion protein of any one of claims 1-8, wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the N- terminus and C-terminus of albumin, or the N-terminus and the C-terminus of the fragment or variant of albumin.

12. The albumin fusion protein of any one of claims 1-8, which comprises a first Therapeutic protein:X, or fragment or variant thereof, and a second Therapeutic protein:X, or fragment or variant thereof, wherein said first Therapeutic protein:X, or fragment or variant thereof, is different from said second Therapeutic protein:X, or fragment or variant thereof.

13. The albumin fusion protein of any one of claims 1-11, wherein the Therapeutic protein:X, or fragment or variant thereof, is separated from the albumin or the fragment or variant of albumin by a linker.

14. The albumin fusion protein of any one of claims 1-11, wherein the albumin fusion protein has the following formula:

R1-L-R2; R2-L-R1; or R1-L-R2-L-R1,

wherein R1 is Therapeutic protein:X, or fragment or variant thereof, L is a peptide linker, and R2 is albumin comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant of albumin.

15. The albumin fusion protein of any one of claims 1-14, wherein the shelf-life of the albumin fusion protein is greater than the shelf-life of the Therapeutic protein:X in an unfused state.

16. The albumin fusion protein of any one of claims 1-14, wherein the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to

albumin, or fragment or variant thereof, is greater than the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

17. The albumin fusion protein of any one of claims 1-14, wherein the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vivo biological activity of the Therapeutic protein:X , or fragment or variant thereof, in an unfused state.

18. An albumin fusion protein comprising a peptide inserted into an albumin comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant thereof.

19. An albumin fusion protein comprising a peptide inserted into an albumin comprising an amino acid sequence selected from the group consisting of:

(a) amino acids 54 to 61 of SEQ ID NO:18;

(b) amino acids 76 to 89 of SEQ ID NO:18;

(c) amino acids 92 to100 of SEQ ID NO:18;

(d) amino acids 170 to 176 of SEQ ID NO:18;

(e) amino acids 247 to 252 of SEQ ID NO:18;

(f) amino acids 266 to 277 of SEQ ID NO:18;

(g) amino acids 280 to 288 of SEQ ID NO:18;

(h) amino acids 362 to 368 of SEQ ID NO:18;

(i) amino acids 439 to 447 of SEQ ID NO:18;

(j) amino acids 462 to 475 of SEQ ID NO:18;

(k) amino acids 478 to 486 of SEQ ID NO:18; and

(l) amino acids 560 to 566 of SEQ ID NO:18.

20. The albumin fusion protein of claims 18 or 19, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the shelf-life of the peptide as compared to the shelf-life of the peptide in an unfused state.

21. The albumin fusion protein of claims 18 or 19, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vitro biological activity of the peptide fused to albumin as compared to the in vitro biological activity of the peptide in an unfused state.

22. The albumin fusion protein of claims 18 or 19 wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vivo biological activity of the peptide fused to albumin compared to the in vivo biological activity of the peptide in an unfused state.

23. An albumin fusion protein comprising a single chain antibody or portion thereof and albumin comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant thereof.

24. The albumin fusion protein of any one of claims 1-23, which is non-glycosylated.

25. The albumin fusion protein of any one of claims 1-23, which is expressed in yeast.

26. The albumin fusion protein of claim 25, wherein the yeast is glycosylation deficient.

27. The albumin fusion protein of claim 25 wherein the yeast is glycosylation and protease deficient.

28. The albumin fusion protein of any one of claims 1-23, which is expressed by a mammalian cell.

29. The albumin fusion protein of any one of claims 1-23, wherein the albumin fusion protein is expressed by a mammalian cell in culture.

30. The albumin fusion protein of any one of claims 1-23, wherein the albumin fusion protein further comprises a secretion leader sequence.

31. A composition comprising the albumin fusion protein of any one of claims 1-30 and a pharmaceutically acceptable carrier.

32. A kit comprising the composition of claim 31.

33. A method of treating a disease or disorder in a patient, comprising the step of administering the albumin fusion protein of any one of claims 1-30.

34. The method of claim 33, wherein the disease or disorder comprises indication:Y.

35. The method of claim 34, wherein the Therapeutic protein:X comprises interferon-alpha, or fragment or variant thereof, and the disease or disorder is selected from the group consisting of: Hairy cell leukemia; Kaposi's sarcoma; genital warts; anal warts; chronic hepatitis B; chronic non-A, non-B hepatitis; hepatitis C; hepatitis D; chronic myelogenous leukemia; renal cell carcinoma; bladder carcinoma; ovarian carcinoma; cervical carcinoma; skin cancer; recurrent respirator papillomatosis; non-Hodgkin's lymphoma; cutaneous T-cell lymphoma; melanoma; multiple myeloma; AIDS; multiple sclerosis; and glioblastoma.

36. A method of treating a patient with a disease or disorder that is modulated by Therapeutic protein:X, comprising the step of administering an effective amount of the

albumin fusion protein of any one of claims 1-30.

37. The method of claim 36, wherein the disease or disorder is indication:Y.

38. The method of claim 37, wherein the Therapeutic protein:X is interferon-alpha, or fragment or variant thereof, and the disease or disorder is selected from the group consisting of: Hairy cell leukemia; Kaposi's sarcoma; genital warts; anal warts; chronic hepatitis B; chronic non-A, non-B hepatitis; hepatitis C; hepatitis D; chronic myelogenous leukemia; renal cell carcinoma; bladder carcinoma; ovarian carcinoma; cervical carcinoma; skin cancer; recurrent respirator papillomatosis; non-Hodgkin's lymphoma; cutaneous T-cell lymphoma; melanoma; multiple myeloma; AIDS; multiple sclerosis; and glioblastoma.

39. A method of extending the shelf life of Therapeutic protein:X comprising the step of fusing the Therapeutic protein:X, or fragment or variant thereof, to albumin or a fragment or variant of albumin sufficient to extend the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, compared to the shelf-life of the Therapeutic protein:X , or fragment or variant thereof, in an unfused state.

40. A nucleic acid molecule comprising a polynucleotide sequence encoding the albumin fusion protein of any one of claims 1-30.

41. A vector comprising the nucleic acid molecule of claim 40.

42. A host cell comprising the nucleic acid molecule of claim 40.

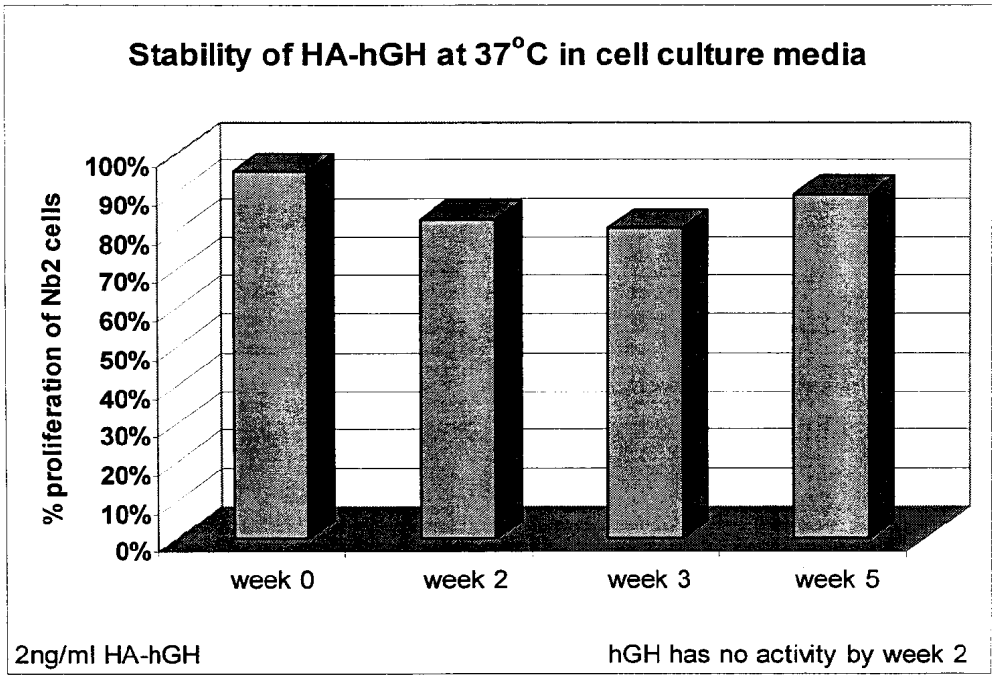

Stability of HA-hGH at 37°C in cell culture media
% proliferation of Nb2 cells
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
week 0
week 2
week 3
week 5
2ng/ml HA-hGH
hGH has no activity by week 2

Figure 1

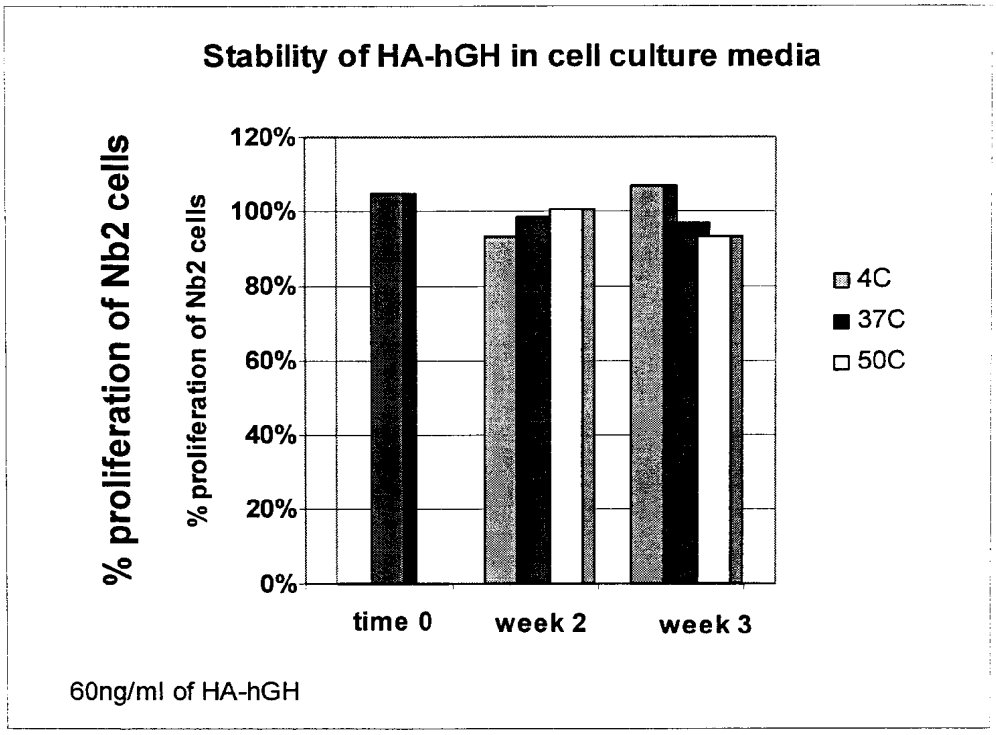

Stability of HA-hGH in cell culture media
% proliferation of Nb2 cells
% proliferation of Nb2 cells
120%
100%
80%
60%
40%
20%
0%
time 0
week 2
week 3
4C
37C
50C
60ng/ml of HA-hGH

Figure 2

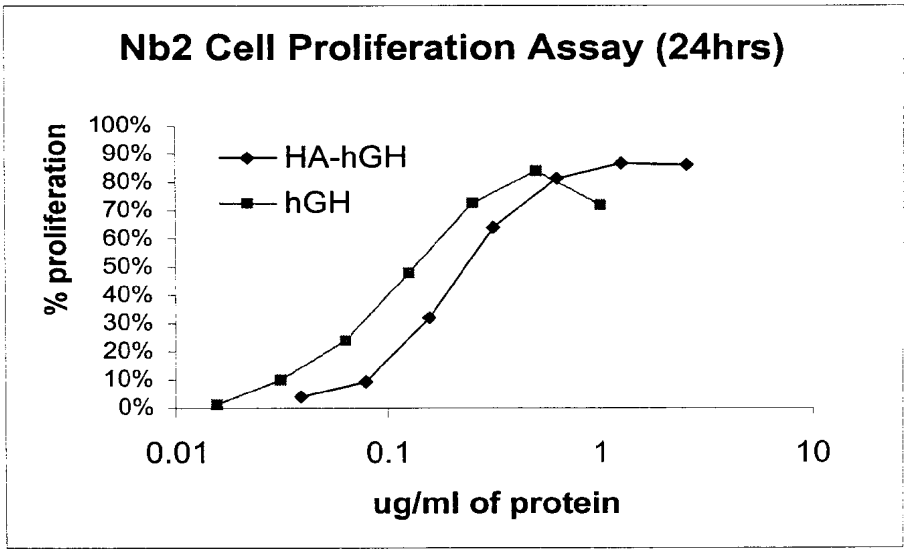


Figure 3A

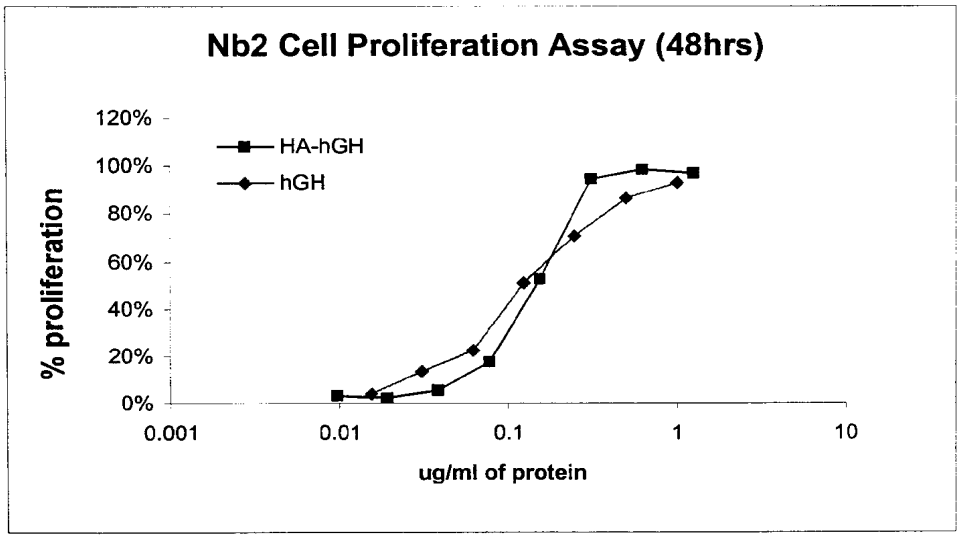


Figure 3B

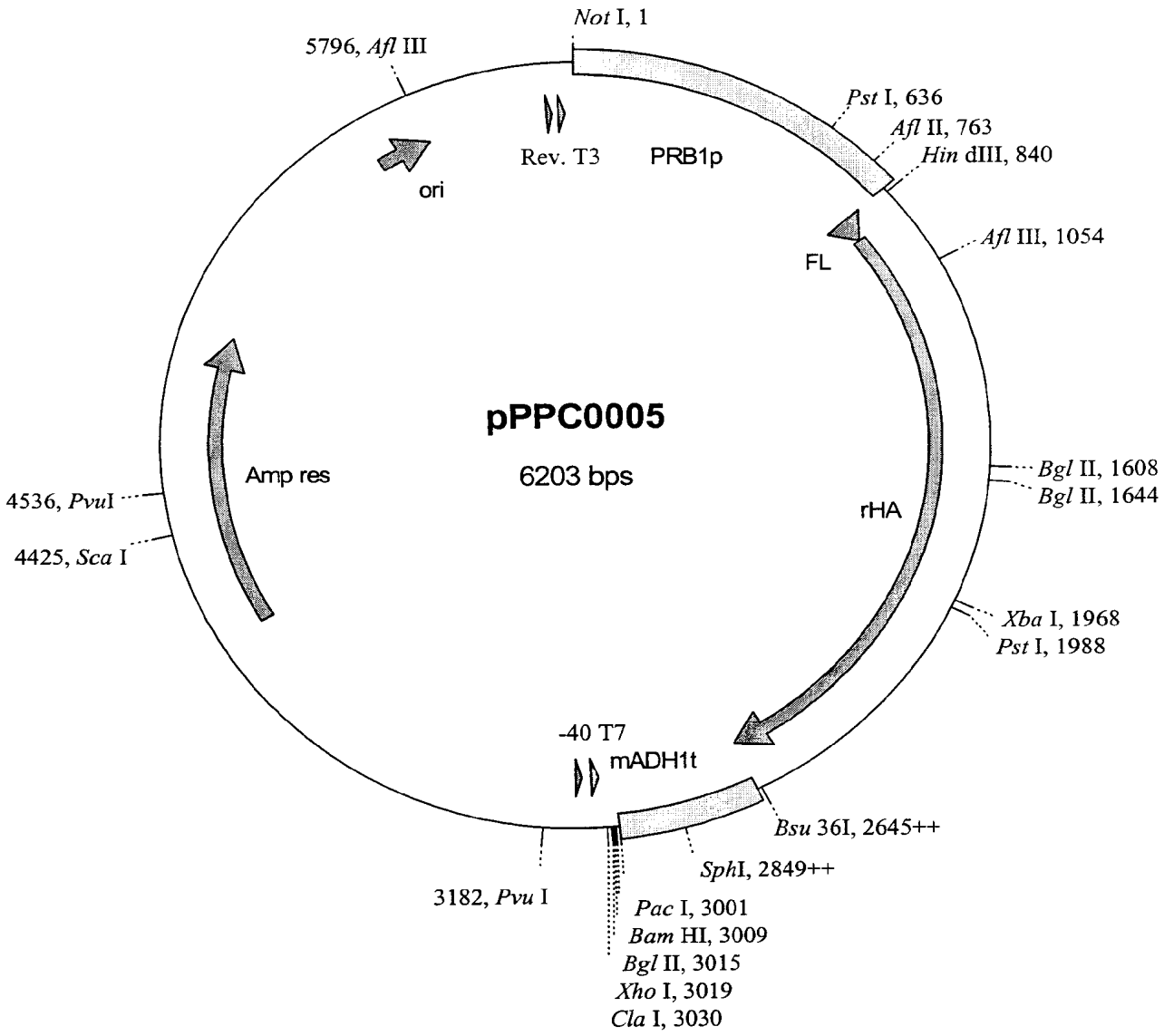

Not I, 1
5796, Afl III
Pst I, 636
Afl II, 763
Hin dIII, 840
Afl III, 1054
Rev. T3
PRB1p
ori
FL
pPPC0005
6203 bps
Amp res
rHA
Bgl II, 1608
Bgl II, 1644
4536, PvuI
4425, Sca I
Xba I, 1968
Pst I, 1988
-40 T7
mADH1t
Bsu 36I, 2645++
SphI, 2849++
3182, Pvu I
Pac I, 3001
Bam HI, 3009
Bgl II, 3015
Xho I, 3019
Cla I, 3030

Figure 4

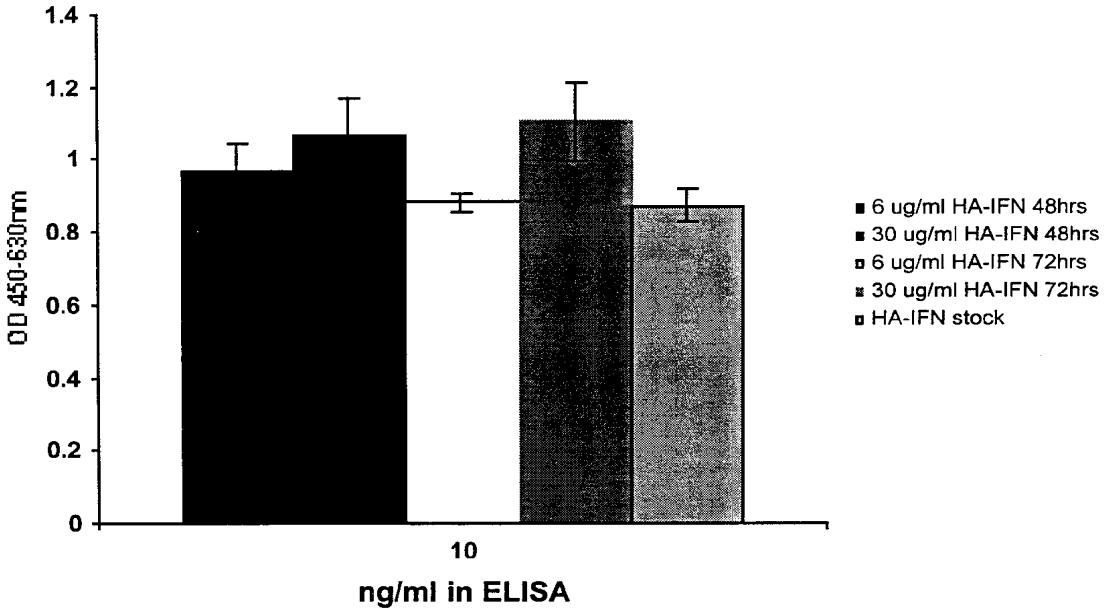

1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD 450-630nm
10
ng/ml in ELISA
6 ug/ml HA-IFN 48hrs
30 ug/ml HA-IFN 48hrs
6 ug/ml HA-IFN 72hrs
30 ug/ml HA-IFN 72hrs
HA-IFN stock

Figure 5

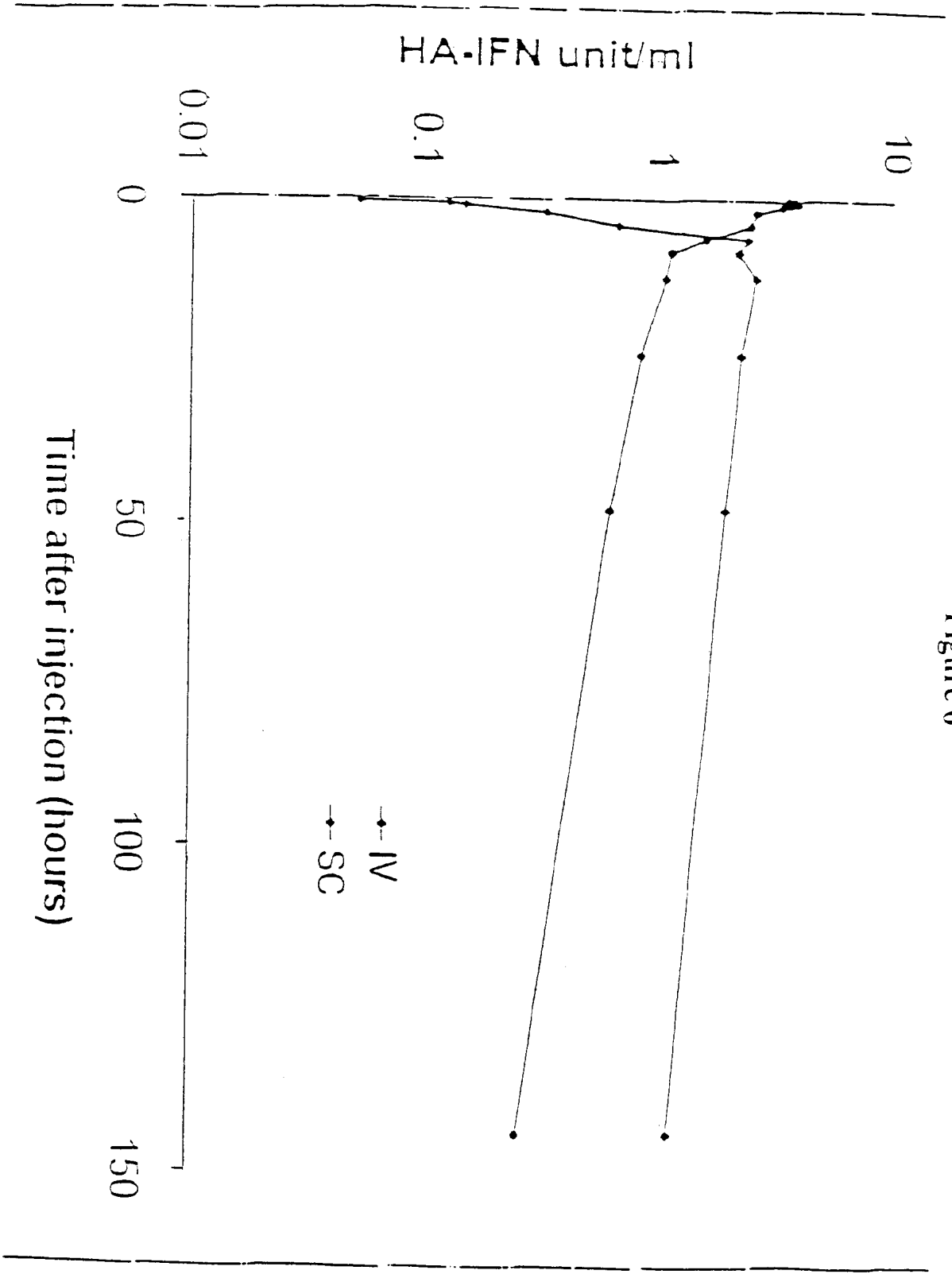

HA-IFN unit/ml
0.01
0.1
1
10
0
50
100
150
Time after injection (hours)
IV
SC

Figure 6

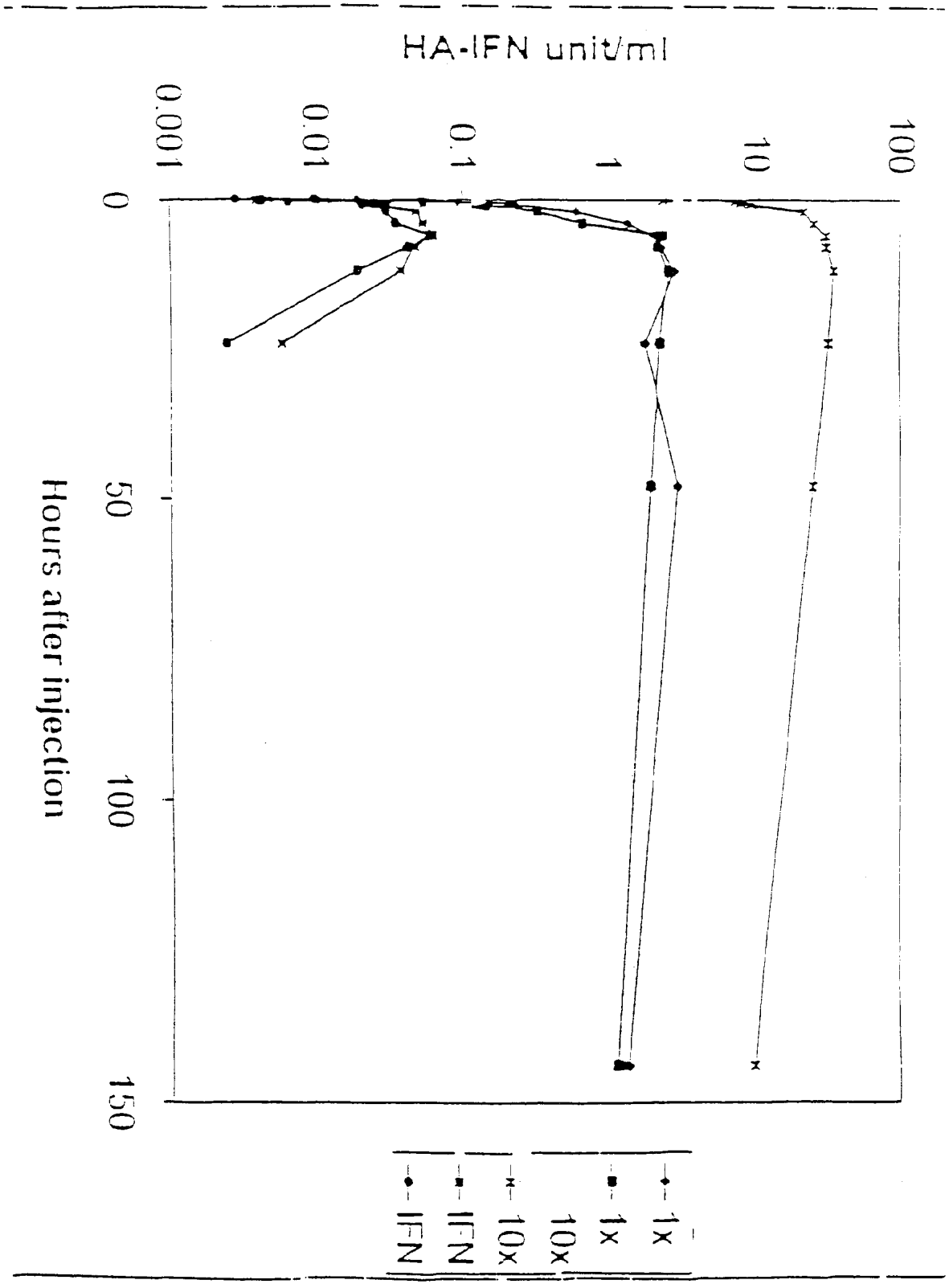
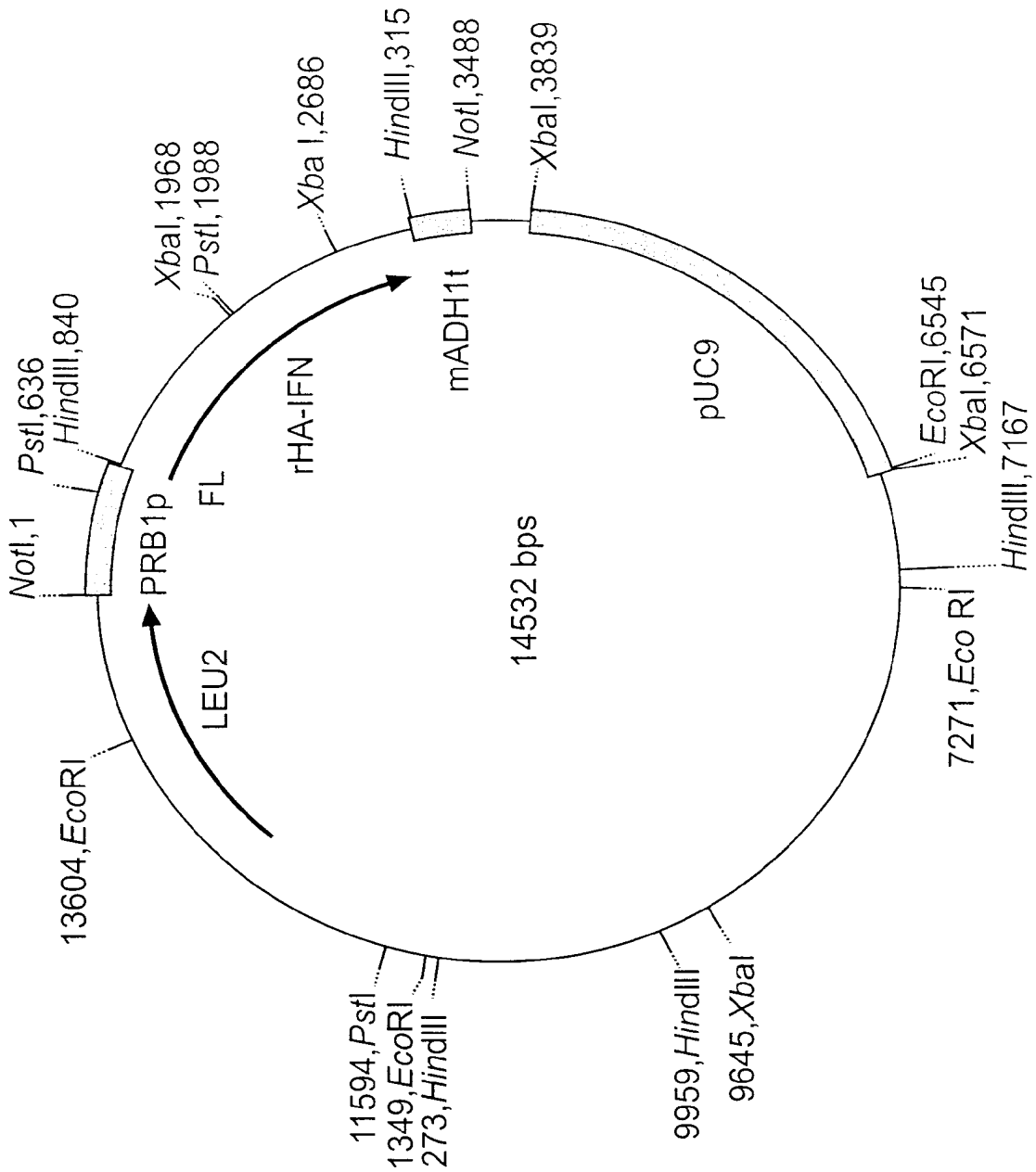

HA-IFN unit/ml
0.001
0.01
0.1
1
10
100
0
50
100
150
Hours after injection
IFN
IFN
10x
10x
1x
1x

Figure 7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PstI,636
HindIII,840
XbaI,1968
PstI,1988
Xba I,2686
HindIII,315
NotI,3488
XbaI,3839
EcoRI,6545
XbaI,6571
HindIII,7167
7271,Eco RI
9645,XbaI
9959,HindIII
11594,PstI
1349,EcoRI
273,HindIII
13604,EcoRI
PRB1p
FL
rHA-IFN
mADH1t
pUC9
LEU2
14532 bps

FIG. 8

Localisation of 'Loops' based on the HA Crystal Structure which could be used for Mutation/Insertion

```
1    DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
          HHHHH HHH     HHH HHHHHHHHHH       HHHHH HHHHHHHHHH

                I                          II                III
51   KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKOEPERNE
     HHHHH             HHHHH HHHHH              HHHH H      HHHH

101  CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
     HHHH                 H HHHHHHHH    HHHHHHHHH HHHHH

                                IV
151  APELLFFAKR YKAAFTECCO AADKAACLLP KLDELRDEGK ASSAKQRLKC
     HHHHHHHHHH HHHHHHHH       HHHHH HHHEHHHHHH HHHHHHHHHH

                                                          V
201  ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
     HHHHH   HH HHHHHHHHHH HH      HHH HHHHHHHHHH HHHHHH  HH

                          VI                  VII
251  LECADDRADL AKYICENODS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
     HHHHHHHHHH HHHHH           HHHHH     HHHHHHH H

301  DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRLA
          HHHH      HHHHHH    HHHHHHH HHHHHH       HHHHHHHH

                    VIII
351  KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
     HHHHHHHHHH       HH    H   HHHHH HHHHHHHHHH HHHHHHH

                                                    IX
401  YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
     HHHHHHHHHH HHHH     H HHHHHHHHHH    HHH       HHHHHHHH

                         X                  XI
451  DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPPCFSA LEVDETYVPK
     HHHHHHHHHH HHHHH      HHHHHHHHH     HHHHHHHH

501  EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
               HHH   HHH HHHHMMEHHH HHH          HHHHHHHH

                    XII
551  FAAFVEKCCK ADDKETCFAE EGKKLVAASQ AALGL
     HHHHHHHH          HHHH HHHHHHHHHH HH
```

Loop		Loop	
I	Val54-Asn61	VII	Glu280-His288
II	Thr76-Asp89	VIII	Ala362-Glu368
III	Ala92-Glu100	IX	Lys439-Pro447
IV	Gln170-Ala176	X	Val462-Lys475
V	His247-Glu252	XI	Thr478-Pro486
VI	Glu266-Glu277	XII	Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

```
                              IV
151   APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH       HHHHH HHHHHHHHHH HHHHHHHHHH

                              IV
151   APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH       HHHHH HHHHHHHHHH HHHHHHHHHH
```

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

$(X)_n$

↓

IV

```
151   APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH       HHHHH HHHHHHHHHH HHHHHHHHHH
```

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

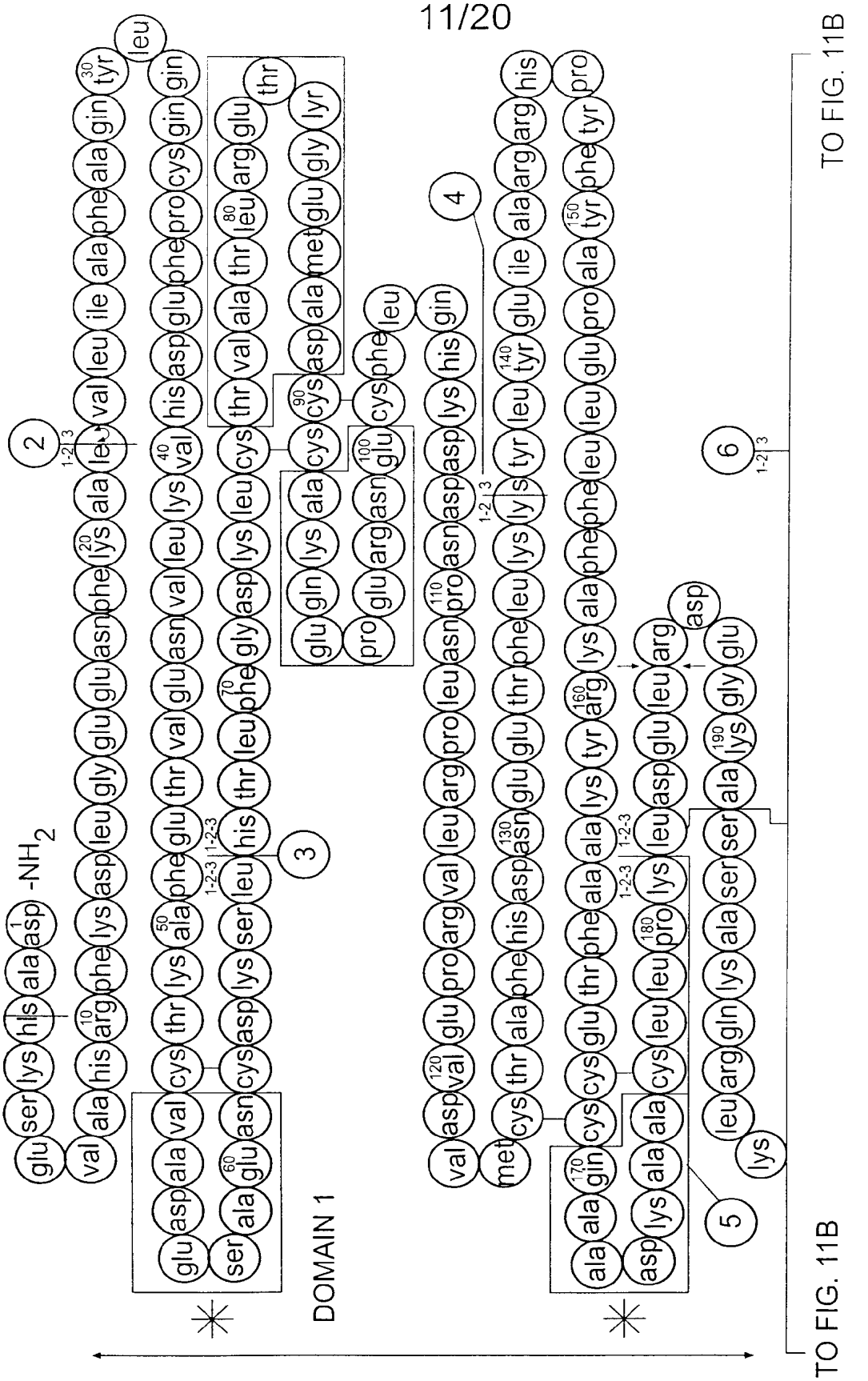

FIG. 11A

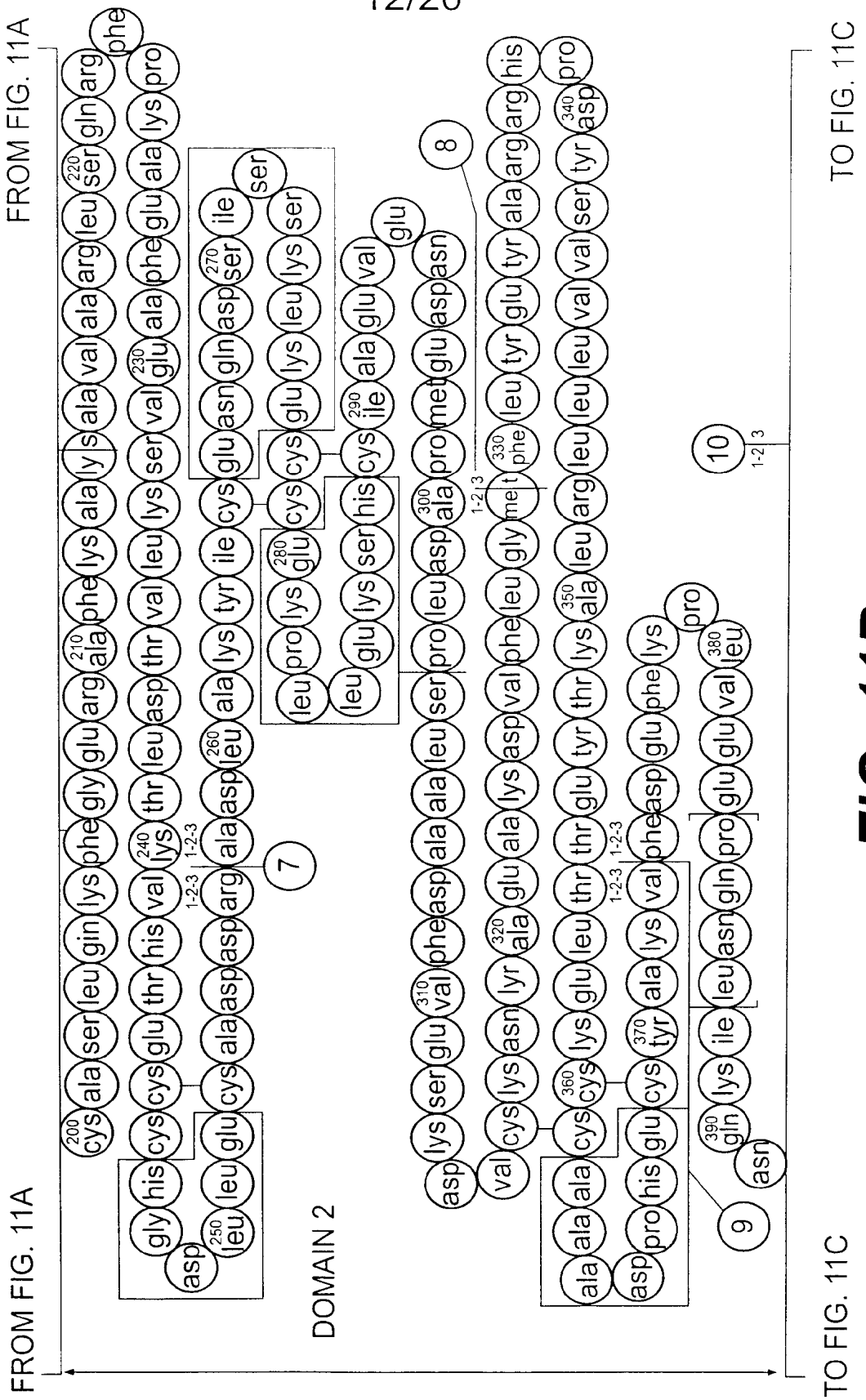
FROM FIG. 11A
FROM FIG. 11A
TO FIG. 11C
TO FIG. 11C
DOMAIN 2
1-2-3
1-2-3
1-2-3
1-2-3
1-2-3
7
8
9
10

FIG. 11B

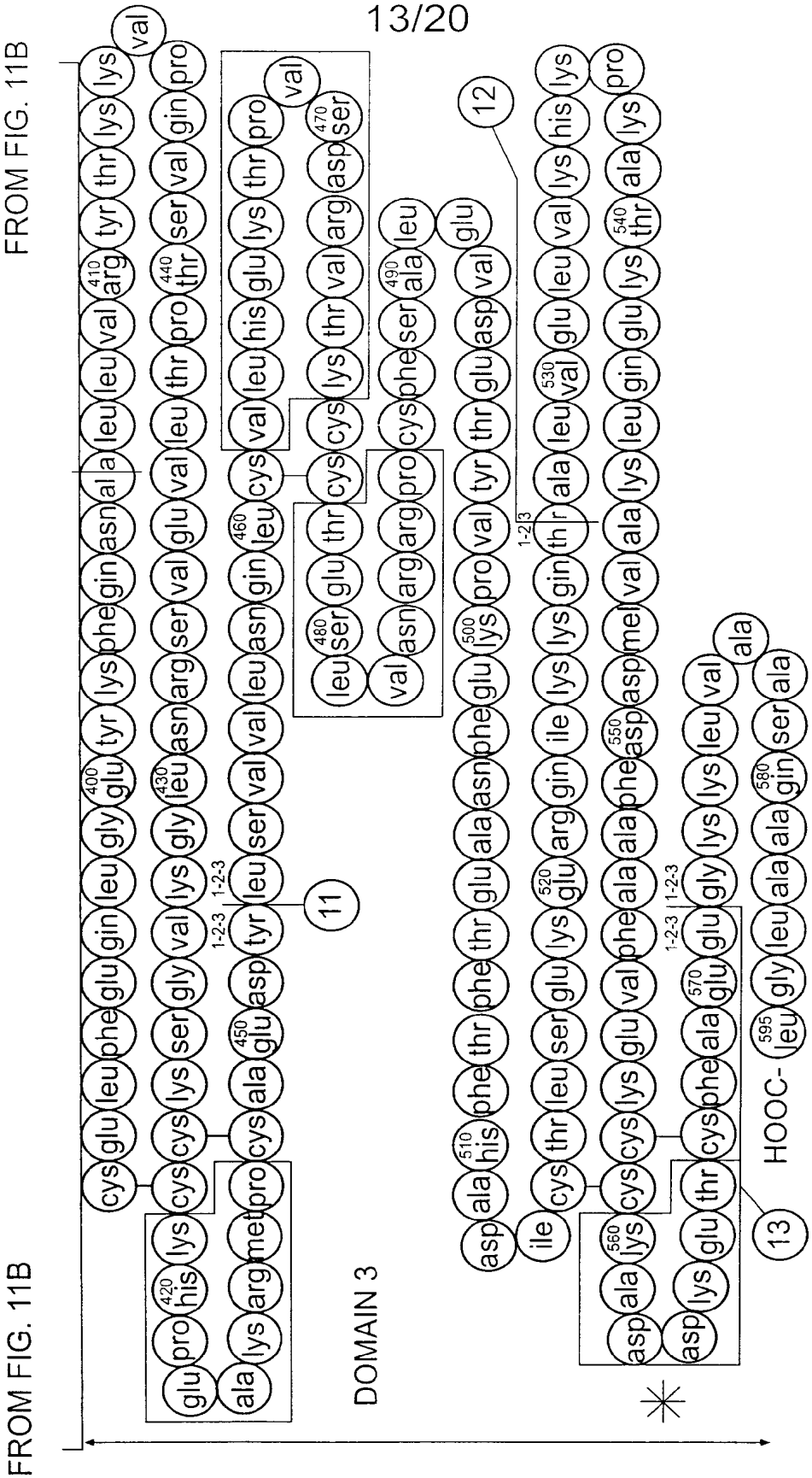

FIG. 11C

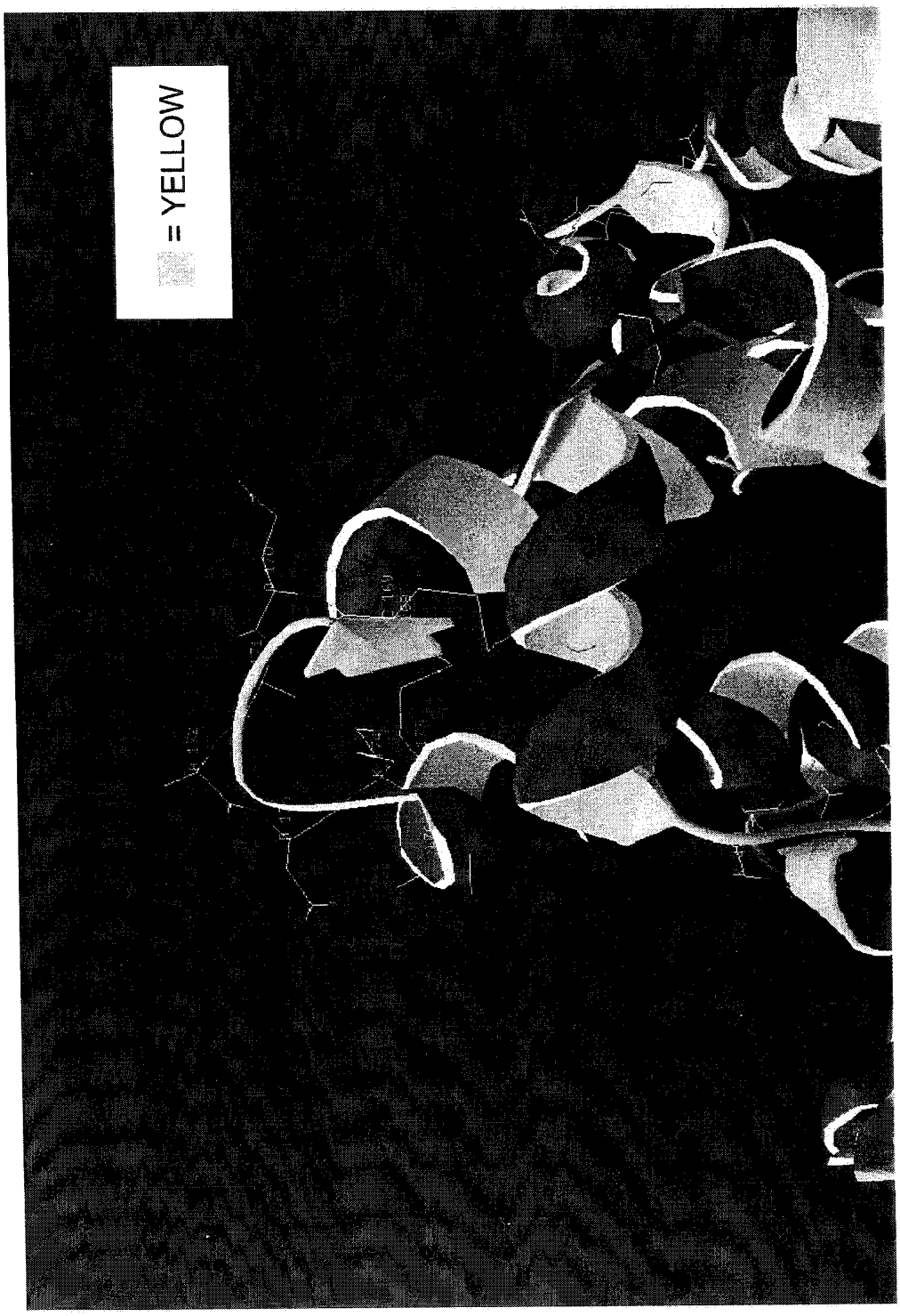

FIG. 12:
LOOP IV GLU170-A176

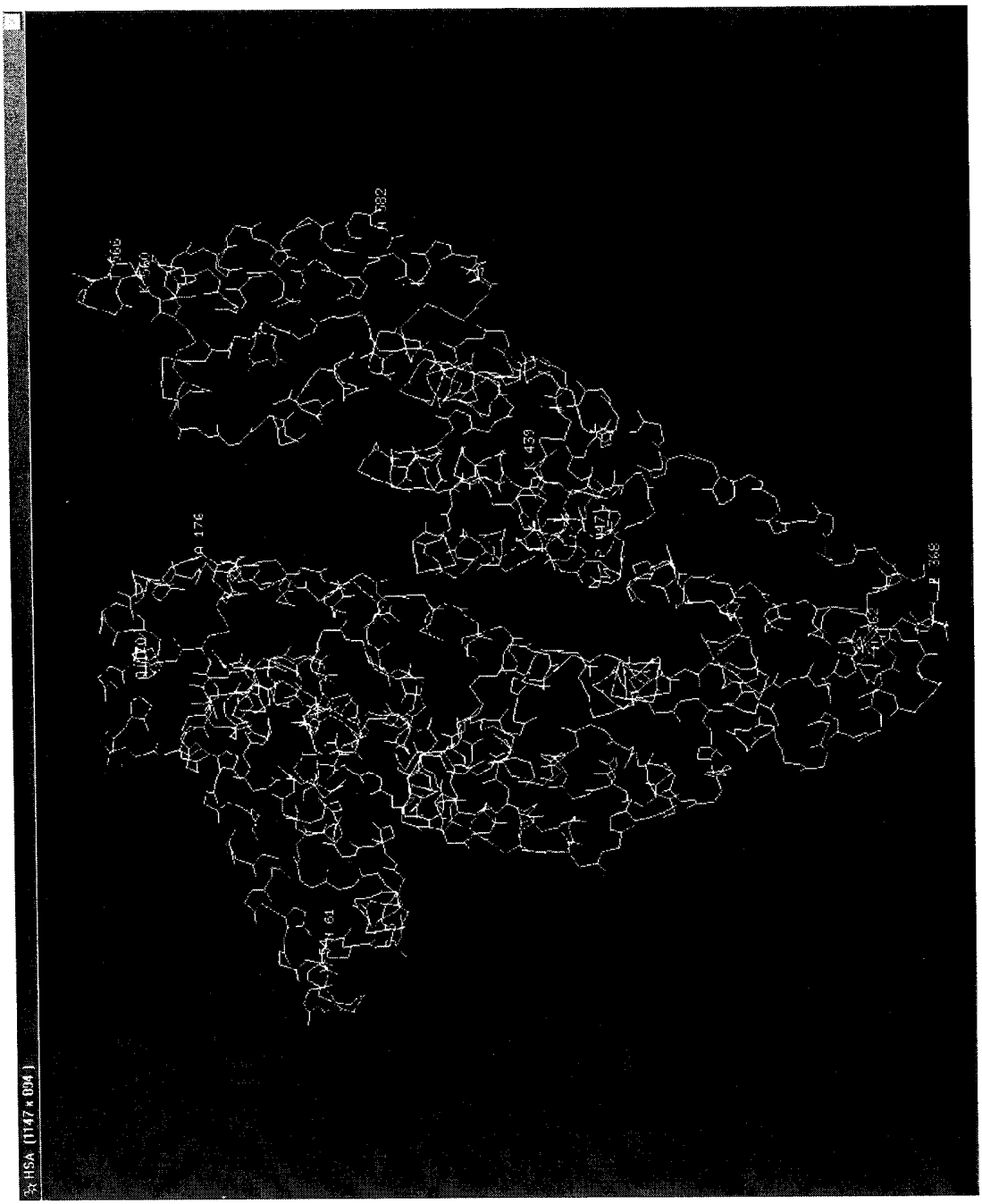

FIG. 13
TERTIARY STRUCTURE OF HA

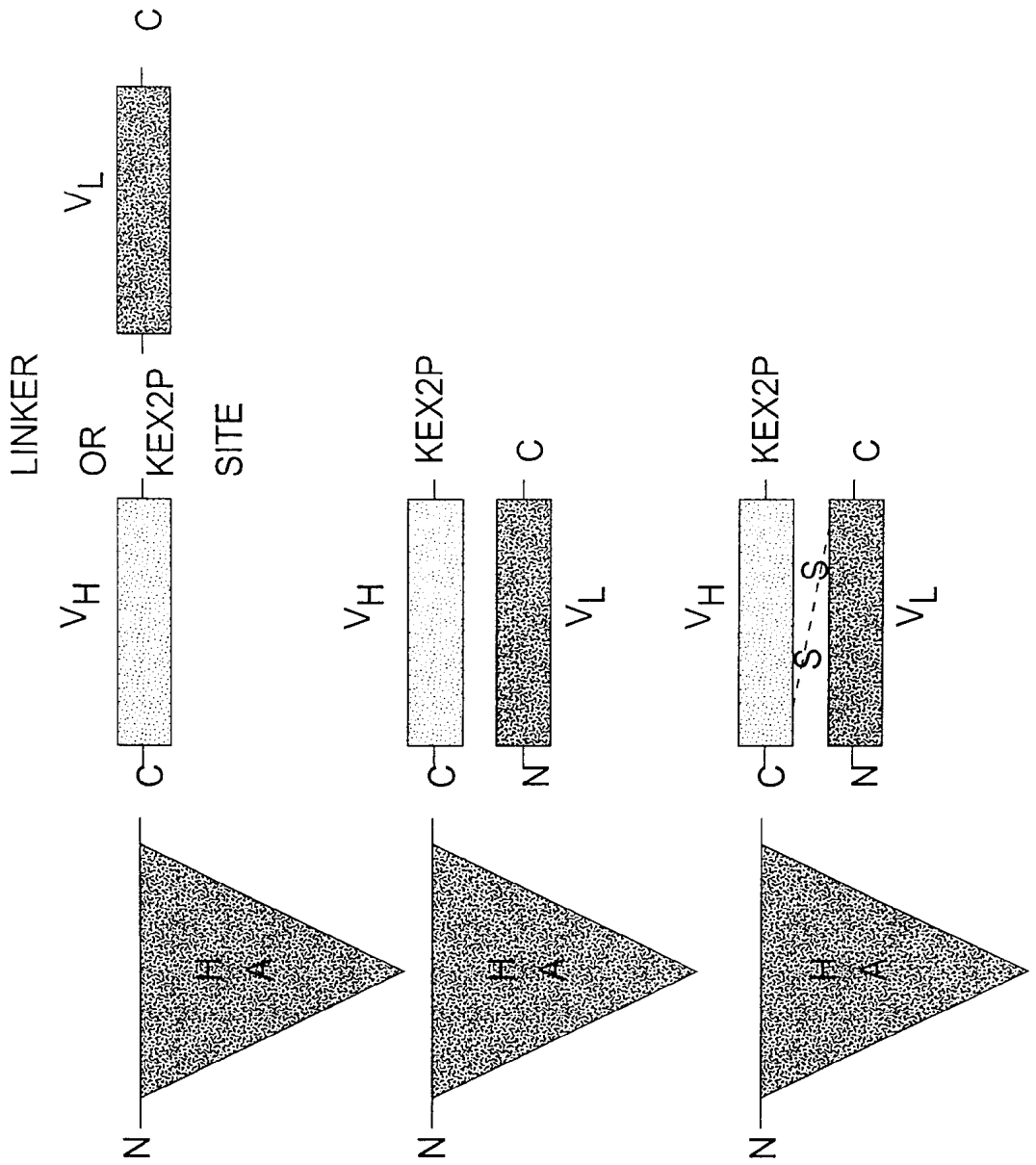

FIG. 14

```
  1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAT TTC AAA  60
  1 D   A   H   K   S   E   V   A   H   R   F   K   D   L   G   E   E   N   F   K    20

 61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
 21 A   L   V   L   I   A   F   A   Q   Y   L   Q   Q   C   P   F   E   D   H   V    40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
 41 K   L   V   N   E   V   T   E   F   A   K   T   C   V   A   D   E   S   A   E    60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
 61 N   C   D   K   S   L   H   T   L   F   G   D   K   L   C   T   V   A   T   L    80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
 81 R   E   T   Y   G   E   M   A   D   C   C   A   K   Q   E   P   E   R   N   E   100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
101 C   F   L   Q   H   K   D   D   N   P   N   L   P   R   L   V   R   P   E   V   120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D   V   M   C   T   A   F   H   D   N   E   E   T   F   L   K   K   Y   L   Y   140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E   I   A   R   R   H   P   Y   F   Y   A   P   E   L   L   F   F   A   K   R   160
```

Figure 15A

```
481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
161 Y   K   A   A   F   T   E   C   C   Q   A   A   D   K   A   A   C   L   L   P   180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K   L   D   E   L   R   D   E   G   K   A   S   S   A   K   Q   R   L   K   C   200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A   S   L   Q   K   F   G   E   R   A   F   K   A   W   A   V   A   R   L   S   220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q   R   F   P   K   A   E   F   A   E   V   S   K   L   V   T   D   L   T   K   240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V   H   T   E   C   C   H   G   D   L   L   E   C   A   D   D   R   A   D   L   260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
261 A   K   Y   I   C   E   N   Q   D   S   I   S   S   K   L   K   E   C   C   E   280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K   P   L   L   E   K   S   H   C   I   A   E   V   E   N   D   E   M   P   A   300

901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D   L   P   S   L   A   A   D   F   V   E   S   K   D   V   C   K   N   Y   A   320
```

Figure 15B

```
961  GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
321  E   A   K   D   V   F   L   G   M   F   L   Y   E   Y   A   R   R   H   P   D   340

1021 TAC TCT GTC GTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
341  Y   S   V   V   L   L   L   R   L   A   K   T   Y   E   T   T   L   E   K   C   360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
361  C   A   A   A   D   P   H   E   C   Y   A   K   V   F   D   E   F   K   P   L   380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
381  V   E   E   P   Q   N   L   I   K   Q   N   C   E   L   F   E   Q   L   G   E   400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
401  Y   K   F   Q   N   A   L   L   V   R   Y   T   K   K   V   P   Q   V   S   T   420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
421  P   T   L   V   E   V   S   R   N   L   G   K   V   G   S   K   C   C   K   H   440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
441  P   E   A   K   R   M   P   C   A   E   D   Y   L   S   V   V   L   N   Q   L   460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
461  C   V   L   H   E   K   T   P   V   S   D   R   V   T   K   C   C   T   E   S   480
```

Figure 15C

```
1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
 481 L   V   N   R   R   P   C   F   S   A   L   E   V   D   E   T   Y   V   P   K   500

1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
 501 E   F   N   A   E   T   F   T   F   H   A   D   I   C   T   L   S   E   K   E   520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
 521 R   Q   I   K   K   Q   T   A   L   V   E   L   V   K   H   K   P   K   A   T   540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680
 541 K   E   Q   L   K   A   V   M   D   D   F   A   A   F   V   E   K   C   C   K   560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740
 561 A   D   D   K   E   T   C   F   A   E   E   G   K   K   L   V   A   A   S   Q   580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782
 581 A   A   L   G   L   *                                 585
```

Figure 15D

SEQUENCE LISTING

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Principia Pharmaceutical Corporation

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<130> PF542PCT

<140> Unassigned
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<151> 2000-04-12

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<151> 2000-12-21

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<220>

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with non-cohesive ends.

<400> 3
gataaagatt cccaac 16

<210> 4
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA fragments
with non-cohesive ends.

<400> 4
aattgttggg aatcttt 17

<210> 5
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA fragments
with non-cohesive ends.

<400> 5
ttaggcttat tcccaac 17

<210> 6
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA fragments
with non-cohesive ends.

<400> 6
aattgttggg aataagcc 18

<210> 7
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> 1)..(19)
<223> invertase leader sequence

<220>
<221> SITE
<222> 20)..(24)
<223> first 5 amino acids of mature human serum albumin

<400> 7
Met Leu Leu Gln Ala Phe Leu Phe Leu Leu Ala Gly Phe Ala Ala Lys
1 5 10 15

Ile Ser Ala Asp Ala His Lys Ser
20

<210> 8
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA fragments with non-cohesive ends.

<400> 8
gagatgcaca cctgagtgag g 21

<210> 9
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA fragments with non-cohesive ends.

<400> 9
gatcctgtgg cttcgatgca cacaaga 27

<210> 10
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA fragments with non-cohesive ends.

<400> 10
ctcttgtgtg catcgaagcc acag 24

<210> 11
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

<400> 11
tgtggaagag cctcagaatt tattcccaac 30

<210> 12
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

<400> 12
aattgttggg aataaattct gaggctcttc c 31

<210> 13
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

<400> 13
ttaggcttag gtggcggtgg atccggcggt ggtggatctt tcccaac 47

<210> 14
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

<400> 14
aattgttggg aaagatccac caccgccgga tccaccgcca cctaagcc 48

<210> 15
<211> 62
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

<400> 15
ttaggcttag gcggtggtgg atctggtggc ggcggatctg gtggcggtgg atccttccca 60
ac 62

<210> 16
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

<400> 16
aattgttggg aaggatccac cgccaccaga tccgccgcca ccagatccac caccgcctaa 60
gcc 63

<210> 17
<211> 1782
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1755)

<400> 17
gat gca cac aag agt gag gtt gct cat cgg ttt aaa gat ttg gga gaa 48
Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
1 5 10 15

gaa aat ttc aaa gcc ttg gtg ttg att gcc ttt gct cag tat ctt cag 96
Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
20 25 30

cag tgt cca ttt gaa gat cat gta aaa tta gtg aat gaa gta act gaa 144
Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
35 40 45

ttt gca aaa aca tgt gtt gct gat gag tca gct gaa aat tgt gac aaa 192
Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
50 55 60

tca ctt cat acc ctt ttt gga gac aaa tta tgc aca gtt gca act ctt 240
Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65 70 75 80

cgt gaa acc tat ggt gaa atg gct gac tgc tgt gca aaa caa gaa cct 288
Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
85 90 95

gag aga aat gaa tgc ttc ttg caa cac aaa gat gac aac cca aac ctc 336
Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
100 105 110

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ccc cga ttg gtg aga cca gag gtt gat gtg atg tgc act gct ttt cat   384
Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
        115                 120                 125

gac aat gaa gag aca ttt ttg aaa aaa tac tta tat gaa att gcc aga   432
Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
    130                 135                 140

aga cat cct tac ttt tat gcc ccg gaa ctc ctt ttc ttt gct aaa agg   480
Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145                 150                 155                 160

tat aaa gct gct ttt aca gaa tgt tgc caa gct gct gat aaa gct gcc   528
Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
                165                 170                 175

tgc ctg ttg cca aag ctc gat gaa ctt cgg gat gaa ggg aag gct tcg   576
Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
            180                 185                 190

tct gcc aaa cag aga ctc aaa tgt gcc agt ctc caa aaa ttt gga gaa   624
Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
        195                 200                 205

aga gct ttc aaa gca tgg gca gtg gct cgc ctg agc cag aga ttt ccc   672
Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
    210                 215                 220

aaa gct gag ttt gca gaa gtt tcc aag tta gtg aca gat ctt acc aaa   720
Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225                 230                 235                 240

gtc cac acg gaa tgc tgc cat gga gat ctg ctt gaa tgt gct gat gac   768
Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
                245                 250                 255

agg gcg gac ctt gcc aag tat atc tgt gaa aat cag gat tcg atc tcc   816
Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
            260                 265                 270

agt aaa ctg aag gaa tgc tgt gaa aaa cct ctg ttg gaa aaa tcc cac   864
Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
        275                 280                 285

tgc att gcc gaa gtg gaa aat gat gag atg cct gct gac ttg cct tca   912
Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
    290                 295                 300

tta gct gct gat ttt gtt gaa agt aag gat gtt tgc aaa aac tat gct   960
Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305                 310                 315                 320

gag gca aag gat gtc ttc ctg ggc atg ttt ttg tat gaa tat gca aga   1008
Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
                325                 330                 335

agg cat cct gat tac tct gtc gtg ctg ctg ctg aga ctt gcc aag aca   1056
Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
            340                 345                 350
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tat gaa acc act cta gag aag tgc tgt gcc gct gca gat cct cat gaa   1104
Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
        355                 360                 365

tgc tat gcc aaa gtg ttc gat gaa ttt aaa cct ctt gtg gaa gag cct   1152
Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
    370                 375                 380

cag aat tta atc aaa caa aac tgt gag ctt ttt gag cag ctt gga gag   1200
Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385                 390                 395                 400

tac aaa ttc cag aat gcg cta tta gtt cgt tac acc aag aaa gta ccc   1248
Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
                405                 410                 415

caa gtg tca act cca act ctt gta gag gtc tca aga aac cta gga aaa   1296
Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
            420                 425                 430

gtg ggc agc aaa tgt tgt aaa cat cct gaa gca aaa aga atg ccc tgt   1344
Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
        435                 440                 445

gca gaa gac tat cta tcc gtg gtc ctg aac cag tta tgt gtg ttg cat   1392
Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
    450                 455                 460

gag aaa acg cca gta agt gac aga gtc aca aaa tgc tgc aca gag tcc   1440
Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465                 470                 475                 480

ttg gtg aac agg cga cca tgc ttt tca gct ctg gaa gtc gat gaa aca   1488
Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
                485                 490                 495

tac gtt ccc aaa gag ttt aat gct gaa aca ttc acc ttc cat gca gat   1536
Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
            500                 505                 510

ata tgc aca ctt tct gag aag gag aga caa atc aag aaa caa act gca   1584
Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
        515                 520                 525

ctt gtt gag ctt gtg aaa cac aag ccc aag gca aca aaa gag caa ctg   1632
Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
    530                 535                 540

aaa gct gtt atg gat gat ttc gca gct ttt gta gag aag tgc tgc aag   1680
Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545                 550                 555                 560

gct gac gat aag gag acc tgc ttt gcc gag gag ggt aaa aaa ctt gtt   1728
Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
                565                 570                 575

gct gca agt caa gct gcc tta ggc tta taacatctac atttaaaagc atctcag 1782
Ala Ala Ser Gln Ala Ala Leu Gly Leu
            580                 585
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<210> 18
<211> 585
<212> PRT
<213> Homo Sapiens

<400> 18

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Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
  1               5                  10                  15

Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
             20                  25                  30

Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
          35                  40                  45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
     50                  55                  60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
 65                  70                  75                  80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
                 85                  90                  95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
            100                 105                 110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
        115                 120                 125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
    130                 135                 140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145                 150                 155                 160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
                165                 170                 175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
            180                 185                 190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
        195                 200                 205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
    210                 215                 220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225                 230                 235                 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
                245                 250                 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
            260                 265                 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
        275                 280                 285
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Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
    290                 295                 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305                 310                 315                 320

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
                325                 330                 335

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
            340                 345                 350

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
        355                 360                 365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
    370                 375                 380

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385                 390                 395                 400

Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
                405                 410                 415

Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
            420                 425                 430

Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
        435                 440                 445

Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
    450                 455                 460

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465                 470                 475                 480

Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
                485                 490                 495

Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
            500                 505                 510

Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
        515                 520                 525

Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
    530                 535                 540

Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545                 550                 555                 560

Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
                565                 570                 575

Ala Ala Ser Gln Ala Ala Leu Gly Leu
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<210> 19
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> primer used to generate XhoI and ClaI
site in pPPC0006

<400> 19
gcctcgagaa aagagatgca cacaagagtg aggttgctca tcgatttaaa gatttgg 57

<210> 20
<211> 58
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> primer used in generation XhoI and ClaI
site in pPPC0006

<400> 20
aatcgatgag caacctcact cttgtgtgca tctcttttct cgaggctcct ggaataag 58

<210> 21
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> primer used in generation XhoI and ClaI
site in pPPC0006

<400> 21
tacaaactta agagtccaat tagc 24

<210> 22
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> primer used in generation XhoI and ClaI
site in pPPC0006

<400> 22
cacttctcta gagtggtttc atatgtctt 29

<210> 23
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<221> Misc_Structure
<223> Synthetic oligonucleotide used to alter restriction
sites in pPPC0007

<400> 23
aagctgcctt aggcttataa taaggcgcgc cggccggccg tttaaactaa gcttaattct 60

<210> 24
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<221> Misc_Structure
<223> Synthetic oligonucleotide used to alter restriction
sites in pPPC0007

<400> 24
agaattaagc ttagtttaaa cggccggccg gcgcgcctta ttataagcct aaggcagctt 60

<210> 25
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> forward primer useful for generation of albumin
fusion protein in which the albumin moiety is N-terminal
of the Therapeutic Protein

<220>
<221> misc_feature
<222> (18)
<223> n equals a,t,g, or c

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<400> 25
aagctgcctt aggcttannn nnnnnnnnnn nn 32

<210> 26
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
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<223> reverse primer useful for generation of albumin fusion protein in which the albumin moiety is N-terminal of the Therapeutic Protein

<220>
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<220>
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<222> (51)
<223> n equals a,t,g, or c

<400> 26
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<210> 27
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer useful for generation of albumin fusion protein in which the albumin moiety is c-terminal of the Therapeutic Protein

<220>
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<221> misc_feature
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<400> 27
aggagcgtcg acaaaagann nnnnnnnnnn nnn 33

<210> 28
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
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<223> reverse primer useful for generation of albumin

fusion protein in which the albumin moiety is c-terminal of the Therapeutic Protein

<220>
<221> misc_feature
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<220>
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<220>
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<222> (49)
<223> n equals a,t,g, or c

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<223> n equals a,t,g, or c

<220>
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<223> n equals a,t,g, or c

<220>
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<222> (52)
<223> n equals a,t,g, or c

<400> 28
ctttaaatcg atgagcaacc tcactcttgt gtgcatcnnn nnnnnnnnnn nn 52

<210> 29
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> signal peptide of natural human serum albumin protein

<400> 29
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1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg
20

<210> 30
<211> 114
<212> DNA
<213> Artificial Sequence

<220>
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<223> forward primer useful for generation of PC4:HSA
albumin fusion VECTOR

<220>
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<222> (5)..(10)
<223> BamHI retsriction site

<220>
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<223> Hind III retsriction site

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<222> (17)..(27)
<223> Kozak sequence

<220>
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<222> (25)..(97)
<223> cds natural signal sequence of human serum albumin

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<223> XhoI restriction site

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<222> (98)..(114)
<223> cds first six amino acids of human serum albumin

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tttagctcgg cttactcgag gggtgtgttt cgtcgagatg cacacaagag tgag 114

<210> 31
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<212> DNA
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PC4:HSA albumin fusion VECTOR

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<222> (12)..(17)
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<222> (15)..(17)
<223> reverse complement of stop codon

<220>
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<222> (18)..(25)
<223> AscI restriction site

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<210> 32
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<212> DNA
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<220>
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<223> reverse primer useful for inserting Therapeutic protein into pC4:HSA vector

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<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> Stanniocalcin signal peptide

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Ala

<210> 35
<211> 22
<212> PRT
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<211> 66
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Gln Gly Lys Asp Asn Gln Cys Val Thr Gly Glu Gly Thr Pro Lys Pro
35 40 45

Gln Ser His Asn Gln Gly Asp Phe Glu Pro Ile Pro Glu Asp Ala Tyr
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Asp Glu
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<210> 37
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<213> Agkistrodon piscivorus

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Leu Cys Cys Asp Gln Cys Lys Phe Met Lys Glu Gly Thr Val Cys Arg
35 40 45

Ala Arg Gly Asp Asp Val Asn Asp Tyr Cys Asn Gly Ile Ser Ala Gly
50 55 60

Cys Pro Arg Asn Pro Phe His
65 70

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 58, line 12.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
11 April 2001	PTA-3276

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☒ This sheet was received by the International Bureau on 16 MAY 2001
Authorized officer	Authorized officer

ATCC Deposit No.: PTA-3276

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: PTA-3276

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 58, line 12

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
11 April 2001	PTA-3277

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).
Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g. "Accession Number of Deposit")*

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Authorized officer	Authorized officer

Revised Form PCT/RO/134 (January 2001) Petro134ep sollist

ATCC Deposit No.: PTA-3277

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

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ATCC Deposit No.: PTA-3277

DENMARK

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SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 58, line 12.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
11 April 2001	PTA-3278

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer	Authorized officer

ATCC Deposit No.: PTA-3278

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: PTA-3278

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 58, line 12.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
11 April 2001	PTA-3279

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer	Authorized officer

ATCC Deposit No.: PTA-3279

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

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UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: PTA-3279

DENMARK

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SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/12009

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :C07K 14/00; C07H 21/04; C12N 15/00; A61K 38/16
US CL : 435/320.1; 514/2; 530/350; 536/23.4
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1; 514/2; 530/350; 536/23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, CAPLUS, various sequence databases
search terms: chimera, fusion, human serum albumin, interferon, cytokine, erythropoietin, SEQ ID No. 18

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 99/66054 A2 (GENZYME TRANSGENICS CORP.) 23 December 1999, see entire document.	1,2,4,6,9,10,12,14,16,17,24,28,30-37,40-42 3,15,25,26,29,39 N
X Y	WO 97/24445 A1 (DELTA BIOTECHNOLOGY LIMITED) 10 July 1997, seen entire document, especially pp. 3-6, 12, 13, and 19-26	1-4,6,9,10,13-17,25,28-34,36,37,39-42 5,7,8,24,26,27,35,38

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 JULY 2001	Date of mailing of the international search report 22 AUG 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BRONWEN M. LOF TERRY J. DEY PARALEGAL SPECIALIST TECHNOLOGY CENTER 1600 Telephone No. (703) [illegible]

Form PCT/ISA/210 (second sheet) (July 1998) *

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/12009

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,705,363 A (IMAKAWA, K.) 6 January 1998, see entire document, especially col. 23, line 44-col. 24, line 56.	1,2,4,5-7,10,25,33-38,40-42 --- 3,14,15,16,17,24,
X	US 5,766,883 A (BALLANCE et al) 16 June 1998, see entire document, especially col.1 ,line 38-54, col. 6, line 53-col 10, line 41, col. 11, line 36-col. 12, line 40.	1,2,4,6,10,13, 14,25,30-34,36,37,40-42